

IN THE SPECIFICATION

Replace the paragraph beginning with "Sequence data" on pages 6-7 of the specification with the following paragraph.

Sequence data

The isolates 87-012, 174F and 145SL were grown on the human lung carcinoma line A549 in 1600 cm² roller bottles. Full CPE was observed after 5-10 days. Supernate was filtered through 0.45 µm cellulose acetate filters (Costar) and the virus was pelleted at 20,000 g for 20 h at 4°C. RNA was isolated from the virus containing pellets using acid guanidinium thiocyanate as described (Chomczynski and Sacchi). Synthesis of cDNA was performed under standard conditions using 1 µg of RNA, AMV reverse transcriptase (Boehringer-Mannheim) and random 14 mer oligonucleotides as primers in a 20 µL reaction. Fragments of the viral 5'UTR were amplified using cardiovirus specific consensus primers: (sense) 5'-GGCCGAAGCCGCTTGAATA-3' (SEM) [SEQ ID NO. 21] and (antisense) 5'-GTGGCTTTTGGCCGAGAG-3' (ATVEM) [SEQ ID NO. 22], both primers modified after the EMCV2 and EMCV1 primers previously reported (Jongen et al. 1993. Ann. Rheum. Dis. 52: 575-578). Cardiovirus sequences were from Dr. a. Palmenberg (personal communication). Amplification conditions were 30 cycles at: 94°C, 30 sec., 50°C, 30 sec., 72°C, 2 min. The amplified fragments were cloned into the pCRII T-vector (In-Vitrogen). The cloned viral sequences were sequenced using A Taq polymerase FS cycle sequencing kit and data

DE 1
was collected on a ABI Prism 310 sequencing machine using M13-21 and M13 reverse primers (Perkin-Elmer). A 1.8 kb fragment extending from the 5'-UTR into the viral polyprotein sequences was obtained by PCR (polymerase chain reaction) amplification of cDNA from the 145SL isolate. The primers were: (sense) 5'-ACAGTGCATTCCACAC-3' (SLJU1) [SEQ ID NO. 23] OR 5'-CCGCTCCACAATAGA-3' (SLJU2) [SEQ ID NO. 24] and (antisense) 5'-GATCTCAGAC-3' (primer 118) [SEQ ID NO. 25]. The SLJU1 and SLJU2 primers are located immediately adjacent to one another and were chosen as consensus primers for the Ljungan isolates of the invention with as little homology as possible to the EMCV and TMEV groups of viruses. The amplification conditions were 30 cycles at: 94°C, 30 sec., 42°C, 1 min, 72°C, 2 min. The antisense primer 118 [SEQ ID NO. 25] yielded similarly sized PCR products with either the SLJU1 [SEQ ID NO. 23] or SLJU2 [SEQ ID NO. 24] as sense primers, but none of the primers yielded PCR fragments when used alone. The sequence of the primer 118 [SEQ ID NO. 25] was previously published (Bauer, D., et al. 1993. Nucl. Acids Res. 21: 4272-4280). The obtained 1.8 kb PCR fragment was cloned and subsequently sequenced as described above.

Replace the paragraph beginning "The strategy chosen" on page 9 of the specification with the following paragraph.

DE 2
The strategy chosen for obtaining additional PCR fragments from the Ljungan virus isolates was a modification of a technique for detecting differentially

E2

expressed mRNAs (Bauer, D., et al. 1993 Nucl. Acids Res. 21: 4272-4280). As a test of this strategy, cDNA from the Ljungan 145SL isolate was amplified using the conditions above, using either the SLJU1 [SEQ ID NO. 23] or the SLJU2 [SEQ ID NO. 24] primer as a sense primer and one of twenty 10-mer oligonucleotides of randomly chosen sequence as "antisense" primer.

Replace the paragraph beginning "If the PCR products obtained" on pages 9-10 of the specification with the following paragraph.

E3

If the PCR products obtained with the SLJU1 [SEQ ID NO. 23] or SLJU2 [SEQ ID NO. 24] primers and a specific 10-mer were similarly size, and none of the primers yielded a product of this sized when used alone in the PCR reaction, the fragment obtained was isolated and cloned. Only one combination of primers satisfied this criterion, namely the SLJU1 [SEQ ID NO. 23] or SLJU2 [SEQ ID NO. 24] primers in combination with the 118 10-mer oligonucleotide [SEQ ID NO. 25], which yielded a 1.8-1.9 kb PCR product. Of this fragment, 819 bp were sequenced from the 3' end. This sequence contained an open reading frame (ORF) of 663 bp in the sense of the viral polyprotein. This ORF was used to search in the Swiss protein data bank using the BLITZ search service from EMBL with the default search parameters. The top 10 scores were picornavirus polyprotein sequences, including 8 cardiovirus sequences. Homology was found over 188 a.a. The relatedness of this segment of the viral polypeptide to previously sequenced cardioviruses is shown in Table 3. A comparative alignment of all

Q3
cardioviruses was made available to us by Dr. A. Palmenberg. In Table 3, the sequence of TMEBeAn was arbitrarily taken as the index strain. For the 12 remaining cardioviruses in the alignment, only differences in amino acid sequence are shown. The alignment of the Ljungan 145SL sequence is similarly represented at the top. Since the BLITZ search algorithm takes into account identical as well as similar amino acids, the latter have been indicated by small type, while differences to TMEBeAn is in capitals as for the other strains in the alignment.

Replace the paragraph beginning "ALIGNMENT OF SEQUENCES" on page 10 with the following paragraph.

ALIGNMENT OF SEQUENCES

Q4
Table 2 shows an alignment of three Ljungan virus isolates (1. 87-012, 2. 174F, 3. 145SL) [SEQ ID NO. 1, 2 and 3 respectively] with published cardiovirus sequences (4. TMEBeAn, 5. Vilyuisk, 6. EMCV) [SEQ ID NO. 5, 6 and 7 respectively]. The aligned sequence starts 29 nt 3' of the end of the poly-C tract in EMCV, and the sequence corresponds to nt 557-808 (approximately) in the different viral genomes. Inserted spaces in the sequences are indicated by a period (.)

After the Abstract, replace Sequence Listing pages 1-13, filed April 16, 2001, with Sequence Listing pages 1-14 submitted herewith.

new seq. list
placed
after spec-